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(54)【発明の名称】　高安定性酸性リン脂質および製造方法

(57)【要約】
【課題】高安定性酸性リン脂質およびその製造方法を提供する。
【解決手段】酸性リン脂質と賦形剤を共存させることにより安定な酸性リン脂質を製造する方法において、０～５０℃の条件下で水に酸性リン脂質と賦形剤を添加し、均質化後、真空乾燥して水分を１％以下とすることを特徴とする高安定性酸性リン脂質の製造方法。

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【特許請求の範囲】

【請求項１】酸性リン脂質と賦形剤を共存させることにより安定な酸性リン脂質を製造する方法において、０～５０℃の条件下で水に酸性リン脂質と賦形剤を添加し、均質化後、真空乾燥して水分を１％以下とすることを特徴とする高安定性酸性リン脂質の製造方法。

【請求項２】酸性リン脂質がホスファチジン酸またはホスファチジルセリンであり、賦形剤が炭水化物、蛋白質、ペプチドおよびアミノ酸からなる群より選択される１種または２種以上であり、賦形剤の量が酸性リン脂質１重量部に対して、０．０１～１００重量部である請求項１記載の高安定性酸性リン脂質の製造方法。

【請求項３】賦形剤である蛋白質が、ラクトアルブミンまたはゼラチンで、賦形剤であるペプチドが卵白ペプチドである請求項２記載の高安定性酸性リン脂質の製造方法。

【請求項４】請求項１～３の製造方法によって得られる酸性リン脂質であって、保存温度４０℃、相対湿度７５％の条件下で６カ月保存中に酸性リン脂質の減少量が１０％以下、過酸化物価の上昇が１以下である高安定性酸性リン脂質。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は、安定性の高い酸性リン脂質およびその製造方法に関する。

【０００２】

【従来の技術】酸性リン脂質は、リン脂質の酸性物であり、例えば、ホスファチジルセリンまたはホスファチジン酸の塩型のものは牛脳や植物から抽出されたり、他のリン脂質を出発原料とする塩基交換反応または加水分解により製造されてきた（特開昭６３－３６１９０号公報、特開昭６３－３６１９１号公報、特開昭６３－３６７９２号公報、特開平２－７９９９０号公報、特開平４－１７１９７６号公報）。しかし、前記に開示された先行技術は、反応後にホスファチジルセリンまたはホスファチジン酸を塩の形で取り出すものであって、酸型のリン脂質が自然界に存在し難く得ることは困難であった。

【０００３】一方、酸性リン脂質については、近年、様々な生理活性が見出されており、特にホスファチジルセリンは学習効果を高めるような脳機能改善効果や、抗ストレス効果等の生理活性が見出されており、医薬品や食品等への応用が期待されている。しかし、これら酸性リン脂質は酸型の状態では化学的に不安定であり、酸化や加水分解等の品質劣化を受けやすかった。このため、現在市場では試薬グレードの酸性リン脂質が冷凍保存状態で流通していた。ところが、酸性リン脂質は、塩型のものと比較し、他の油脂との相溶性に優れ、製品加工を行いやすい利点があり、塩型とは異なる生理活性を有することから、常温で、高安定性酸性リン脂質を製造する方法が望まれていた。

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【０００４】特開昭５４－１２６２０６号公報には、ホスファチジルコリン等のリン脂質含有油脂に　例えば、オクタデシルアクリルアミドーＮ，Ｎージメチルアクリルアミドの共重合体を添加して、リン脂質の褐変を抑える方法が開示されているが、この方法で、酸性リン脂質の酸化、加水分解が抑制されるか否かについては記載がない。特開平８－５３６４３号公報には、アルキルアクリルアミド、Ｎ－置換アクリルアミドからなる水溶性高分子で被覆することによるリン脂質等の膜の安定化技術が開示されている。しかし、この方法で、酸性リン脂質の酸化、加水分解が抑制されるか否かについては記載がない。また、この技術は、膜の安定化技術であり、バルクの酸性リン脂質には適用できないなど問題があった。

【０００５】特表平８－５０５４０３号公報、特表平１１－５０２８５１号公報では、サイトカイン等の蛋白質を用いて、リン脂質を含む疎水性脂質を賦形剤として添加し、蛋白質を安定化する技術が開示されている。しかし、この方法で、酸性リン脂質の酸化、加水分解が抑制されるか否かについては記載がない。また、この技術は、蛋白質の安定化技術であり、酸性リン脂質には適用できない。

【０００６】

【発明が解決しようとする課題】本発明の目的は、常温で、安定性のある酸性リン脂質およびその製造方法を提供することにある。

【０００７】

【課題を解決するための手段】本発明者らは、前記問題点に鑑み鋭意検討した結果、特定の条件下に酸性リン脂質と賦形剤とを配合すると、常温でも、酸性リン脂質が著しく安定化する知見を得て、本発明を完成させるに至った。即ち、本発明は、次の［１］～［４］である。

【０００８】［１］　酸性リン脂質と賦形剤を共存させることにより安定な酸性リン脂質を製造する方法において、０～５０℃の条件下で水に酸性リン脂質と賦形剤を添加し、均質化後、真空乾燥して水分を１％以下とすることを特徴とする高安定性酸性リン脂質の製造方法。

【０００９】［２］　酸性リン脂質がホスファチジン酸またはホスファチジルセリンであり、賦形剤が炭水化物、蛋白質、ペプチドおよびアミノ酸からなる群より選択される１種または２種以上であり、賦形剤の量が酸性リン脂質が１重量部に対して、０．０１～１００重量部である前記［２］の高安定性酸性リン脂質の製造方法。

【００１０】［３］　賦形剤である蛋白質が、ラクトアルブミンまたはゼラチンで、賦形剤であるペプチドが卵白ペプチドである前記の［１］の高安定性酸性リン脂質の製造方法。

【００１１】［４］　前記の［１］～［３］の製造方法によって得られる酸性リン脂質であって、保存温度４０℃、相対湿度７５％の条件下で６カ月保存中に酸性リン脂質の減少量が１０％以下、過酸化物価の上昇が１以下

である高安定性酸性リン脂質。

【００１２】

【発明の実施の形態】本発明に用いるリン脂質は、例えば、レシチン、ホスファチジルコリン、ホスファチジルエタノールアミン、ホスファチジルグリセロール、リゾレシチン等が挙げられる。なかでもホスファチジルセリン、ホスファチジン酸が好ましく挙げられる。用いるリン脂質は、天然資源から抽出したもの、あるいは合成したものの如何を問わず使用できる。また、市販のもの、または公知の方法で調製したものを使用しても良い。例えば、大豆、脱脂大豆、卵黄等から抽出したものを単独で用いてもよいし２種以上組み合わせて用いてもよい。また、動物、植物等の起源については特に限定されない。例えば、大豆レシチン、脱脂大豆レシチン、卵黄レシチン、ホスファチジルコリン、ホスファチジルエタノールアミン、ホスファチジルグリセロール、リゾレシチン等から塩基交換反応もしくは加水分解により得られたものを使用することも可能である。本発明に用いる酸性リン脂質としては、塩型のリン脂質を酸で処理することによって得られる。前記の酸としては、例えば、ギ酸、酢酸、コハク酸、リンゴ酸、フマル酸、マレイン酸、クエン酸、塩酸、硫酸、硝酸、リン酸、フィチン酸等が挙げられる。　これらの中から選ばれる１種または２種以上を組み合わせて用いることができる。陽イオンの除去効果の点から塩酸、硫酸、硝酸、リン酸の無機酸、フィチン酸等の有機酸を使用することがより好ましい。

【００１３】また、緩衝液溶液、例えば、ギ酸－ギ酸ナトリウム緩衝液、グリシン－塩酸緩衝液、酢酸－酢酸ナトリウム緩衝液、クエン酸－クエン酸ナトリウム緩衝液、リン酸緩衝液等を所定のｐＨに調整して用いることがより望ましい。酸または緩衝液の添加量は、塩型リン脂質１ｍｏｌに対し、酸を１～１００ｍｏｌ、好ましくは１５～６０ｍｏｌ、より好ましくは２０～５０ｍｏｌに設定する。添加量が１ｍｏｌ未満では酸の効果が十分でなく、高純度酸性リン脂質を製造することができない。また、酸の添加量が１００ｍｏｌを越えると、加水分解等の副反応が生じるので望ましくない。反応系に添加する酸の温度は０～５０℃、好ましくは５～３０℃、より好ましくは７～２０℃である。温度が０℃未満では酸の粘度が上昇したり凝固するため取り扱いが難しくなる。また、酸の温度が５０℃を越えると、加水分解等の副反応が生じるので望ましくない。また例えば酸性リン脂質の市販品としては、牛脳由来ホスファチジルセリン（純度９８％：シグマ社製品）、大豆由来ホスファチジルセリン（純度９８％：シグマ社製品）、ジパルミトイルホスファチジン酸（純度９９％：シグマ社製品）等が好ましいものとして挙げられる。

【００１４】本発明に用いるリン脂質の構成脂肪酸は、同一または異種であり、炭素数８～２４の飽和または不飽和の脂肪酸である。例えば、カプロン酸、カプリン酸、ラウリン酸、ミリスチン酸、ステアリン酸、ベヘン酸、アラキジン酸、パルミトオレイン酸、オレイン酸、リノール酸、αおよびγ－リノレン酸、エルシン酸、アラキドン酸、エイコサペンタエン酸、ドコサヘキサエン酸、テトラコサテトラエン酸等が挙げられる。なかでも、オレイン酸、リノール酸、エイコサペンタエン酸、ドコサヘキサエン酸が生理活性の面から好ましい。

【００１５】本発明に用いる賦形剤としては、例えば、炭水化物、蛋白質、ペプチドおよびアミノ酸が挙げられる。前記の炭水化物としては、例えば、澱粉、デキストリン、セルロース及びその加水分解物、ブドウ糖、果糖などの単糖類、マルトース、乳糖などの二糖類、アラビアゴム、キサンタンガム、ローカストビーンガム、プルラン等の多糖類等が挙げられる。これらの１種または２種以上を組み合わせて用いてもよい。例えば、市販品として、ラクトース（ラブリノ社製品）、ＳＤ－２０（シクロデキストリン：塩水港精糖（株）製品）、パインデックス（デキストリン：松谷化学（株）製品）等が好ましい物として挙げられる。

【００１６】前記の蛋白質としては、動物および植物系の蛋白質が用いられる。動物性蛋白質としては、例えば、カゼイン、コラーゲン、ホエイ蛋白質、卵蛋白質、魚蛋白質等が挙げられる。またさらに、植物性蛋白質としては、大豆蛋白質、小麦蛋白質、トウモロコシ蛋白質等が挙げられる。前記のペプチドとしては、前記の蛋白質の分解物またはその塩が挙げられ、例えば、卵白ペプチド、魚ペプチド、乳蛋白ペプチド等が挙げられる。前記のアミノ酸としては、前記のペプチドの分解物、あるいは各種アミノ酸を使用することができる。この中でも供給安定性の面から、ゼラチン、卵白ペプチド、ラクトアルブミン、カゼイネート等が好ましく挙げられる。例えば、市販品として、低分子コラーゲンパウダーＳＰＨ（雪印食品（株）製品）、ラクプロダン８０（ラクトアルブミン：ＭＤフーズイングレディエンツジャパン（株）製品）、マグネシウムカゼイネートＳ（ＤＭＶジャパン（株）製品）、ＣＥ９０Ｆ（ホエーペプチド：ＤＭＶジャパン（株）製品）、ＥＰ－３（卵白ペプチド：キューピー（株）製品）等が好ましいものとして挙げられる。

【００１７】本発明で用いる賦形剤の使用量は、原料となる酸性リン脂質１重量部に対して０．０１～１００重量部、好ましくは、０．２～５０重量部、より好ましくは０．５～１０重量部である。賦形剤の添加量を酸性リン脂質１重量部に対して１００重量部より多くしても、それに見合うだけの著しい安定化の向上効果が認められず、０．０１重量部未満では賦形剤による安定化効果が得られない。

【００１８】本発明を実施する形態は、原料となる酸性リン脂質１重量部に対して０．０１～１００重量部の範囲の賦形剤を攪拌槽中にて水に溶解させ、次いで酸性リ

ン脂質の粉末を添加して均質化させることにより、酸性リン脂質を賦形剤で包含させる。包含は、０～５０℃の範囲で行う。５０℃より高くなると、賦形剤である蛋白質の変性を生じることや、酸性リン脂質の分解を伴う、０℃より低くなると水相が凝固してしまう。包含を行う際にはホモゲナイザー、ポリトロン、マイクロフルイダイザー、ホモミキサー等の均質機を併用することができる。なお、反応は窒素還流下で行うことによりリン脂質に含まれる脂肪酸の劣化を防ぐことができる。その後、真空ベルト乾燥機、真空凍結乾燥等の真空乾燥機により乾燥し、最終製品中の水分を１％以下とする。

【００１９】本発明の方法で製造した酸性リン脂質は、保存温度４０℃、相対湿度７５％の条件下で６カ月保存中に酸性リン脂質の減少量が１０％以下、過酸化物価の上昇が１以下を達成できる安定化を著しく向上させたものである。

【００２０】

【発明の効果】本発明の高安定性酸性リン脂質の製造方法は、特定の条件下で、原料の酸性リン脂質と特定の賦形剤を添加して、安定化を図るもので、０～５０℃の条件下で水に酸性リン脂質と賦形剤を添加し、均質化後、真空乾燥して水分を１％以下とする簡単な方法により安定化した酸性リン脂質を得ることができる。様々な生理活性を有する酸性リン脂質を安定化することにより、長期間、品質を維持することができ、工業的に、健康食品や医薬品等へ応用することができる。

【００２１】

【実施例】本発明を実施例に基づいて説明する。用いた測定方法、評価方法を示す。

１．リン脂質の定量

高速液体クロマトグラフィー（ギルソン社製、機種モデル３０３）を用いて行った。固定相にはシリカゲルカラム（径４．６ｍｍ×長さ２５０ｍｍ）を用い、また移動相にはアセトニトリル：メタノール：１０ｍＭリン酸二水素アンモニウム＝６１２：２８９：１００の混合溶媒を用い検出は、紫外線ＵＶ波長２０２ｎｍにおける吸収を測定することにより定量した。

２．過酸化物価（ＰＯＶ）の測定

酸化の指標となるＰＯＶは日本油化学協会制定の基準油脂分析試験法２．４．１２－８６に準じて測定した。

３．水分の測定

試料中の水分は、カールフィッシャー水分計、三菱化学（株）製、機種ＣＡ－０６）を用いて測定した。

４．安定性の評価方法

安定性の評価は、粉末試料を開放状態で、４０℃、相対湿度７５％の恒温槽内で６ヶ月間保存後、酸性リン脂質の含量を高速液体クロマトグラフィー（ＨＰＬＣ）で測定し、その残存率を求めた。

５．酸化安定性

前記の方法により初期および粉末試料を開放状態で、４０℃、相対湿度７５％の恒温槽内で６ヶ月間保存後、過酸化物価（ＰＯＶ）の測定を行った。

【００２２】実施例１

ラクトアルブミン０．０５ｇと水３ｇを混合し超音波洗浄機で撹拌し溶解させ、次いで酸性の牛脳由来ホスファチジルセリン（純度９８％：シグマ社品）０．５ｇを添加後、窒素置換を行いながら３０℃でポリトロン（キネマチカ社製）を用いて１５分間均質化した。均質化後に真空凍結乾燥を行い、粉末０．５４ｇを得た。試料の水分を前記の方法により測定した結果、０．２％であった。

【００２３】実施例２

シクロデキストリン５００ｇを水３０００ｇに入れ、プロペラ撹拌機で溶解し、次いで酸性の大豆由来ホスファチジルセリン（純度９８％：シグマ社品）５００ｇを添加後、１０℃でホモミキサーにて窒素置換を行いながら１５分間均質化した。反応後に真空ベルト乾燥を行い、粉末９９０ｇを得た。同様にして水分を測定した結果、０．１％であった。

【００２４】実施例３

カゼインナトリウム２１４０ｇと水５０００ｇをホモミキサーにて撹拌し溶解させ、次いで酸性のジパルミトイルホスファチジン酸（純度９９％：シグマ社品）５００ｇを添加後、１℃でホモミキサーにより１５分間均質化した。均質化後に凍結乾燥を行い、粉末２５８０ｇを得た。試料の水分を測定した結果、０．３％であった。

【００２５】実施例４

ゼラチン１００ｇと水８００ｇを５０℃に加温後プロペラ撹拌により溶解させ、４５℃に冷却した後に大豆由来ホスファチジルセリン（純度９８％：シグマ社品）１．９ｇを添加後、窒素置換を行い、ソニファイヤー（ブランソン社製）により１５分間均質化した。反応後に凍結乾燥を行い、粉末１０１ｇを得た。試料の水分を同様に測定した結果、０．１％であった。

【００２６】比較例１

ラクトアルブミン０．０５ｇと水３ｇを混合し超音波洗浄機で撹拌し溶解させ、次いで酸性牛脳由来ホスファチジルセリン（純度９８％：シグマ社製品）０．５ｇを添加後、窒素置換を行いながら３０℃でポリトロン（キネマチカ社）を用いて１５分間均質化した。均質化後に温風乾燥を行い、粉末０．４８ｇを得た。試料の水分を同様にして測定した結果、３．０％であった。

【００２７】比較例２

シクロデキストリン２ｇを水３０００ｇに入れ、プロペラ撹拌機で溶解し、次いで酸性の大豆由来ホスファチジルセリン（純度９８％：シグマ社品）５００ｇを添加後、１０℃でホモミキサーにて窒素置換を行いながら１５分間均質化した。反応後にスプレードライを行い、粉末４５９ｇを得た。試料の水分を同様にして測定した結果、５．０％であった。

【００２８】比較例３
カゼインナトリウム２１４０ｇと水５０００ｇをホモミキサーにて撹拌し溶解させ、次いで酸性のジパルミトイルホスファチジン酸（純度９９％：シグマ社製品）５００ｇを添加後、６０℃でホモミキサーにより１５分間均質化した。均質化後に温風乾燥を行い、粉末２２５０ｇを得た。試料の水分を同様にして測定した結果、１０％であった。

【００２９】比較例４
ゼラチン１００ｇと水８００ｇを５０℃に加温後プロペラ撹拌により溶解させ、４５℃に冷却した後に酸性大豆由来ホスファチジルセリン（純度９８％：シグマ社製品）１．９ｇを添加後、さらにプロペラ撹拌により１５分間撹拌した。反応後にスプレードライを行い、粉末８９ｇの粉末を得た。試料の水分を同様にして測定した結果、３．５％であった。

【００３０】試験例１
実施例１～４、比較例１～４で得られた粉末を４０℃、＊相対湿度７５％の恒温槽内で６ヶ月間保存後、ホスファチジルセリンまたはホスファチジン酸の残存量をＨＰＬＣにて測定し、下記に示す計算式で残存率を求めた。
残存率（％）＝（６ヶ月後の酸性リン脂質含量／初期の酸性リン脂質含量）×１００その結果を表１および２に示す。

【００３１】試験例２
実施例１～４および比較例１～４で得られた粉末を４０℃、相対湿度７５％の恒温槽内で６ヶ月間保存後、ＰＯＶの測定を行った。初期値および６カ月後のＰＯＶを表１および２に示した。

【００３２】試験例３
原料のホスファチジルセリン及びホスファチジン酸を前記の試験例の方法と同様にして安定性を評価した。結果を比較例５～７として表３に示す。

【００３３】
【表１】

表1

		実施例 1	2	3	4
仕込み	原料 酸型リン脂質量（g）	牛脳ＰＳ ０．５ｇ	大豆ＰＳ ５００ｇ	ＰＡ ５００ｇ	大豆ＰＳ １．９ｇ
	賦形剤 量（g）	ﾗｸﾄｱﾙﾌﾞﾐﾝ ０．０５ｇ	ｼｸﾛﾃﾞｷｽﾄﾘﾝ ５００ｇ	ｶｾﾞｲﾝﾅﾄﾘｳﾑ ２１４０ｇ	ゼラチン １００ｇ
処理	均質化処理の有無 温度（℃）	あり ３０	あり １０	あり １	あり ４５
	時間（分）	１５	１５	１５	１５
	乾燥方法	真空凍結乾燥	真空ﾍﾞﾙﾄ乾燥	真空凍結乾燥	真空ﾍﾞﾙﾄ乾燥
結果	収量g 収率（％）	０．５４ ９８．２	９９０ ９９．０	２５８０ ９９．７	１０１ ９９．２
	水分（％）	０．２	０．１	０．３	０．１
	残存率（％）	９９	９６	９８	９８
	初期ＰＯＶ* 経時試験後ＰＯＶ	０．１ ０．３	０．３ ０．５	０．１ ０．４	０．３ ０．４

【００３４】なお表中に用いた略号は次のとおりである。
ＰＳ；ホスファチジルセリン（酸型）
ＰＡ；ホスファチジン酸。
また、ＰＯＶの単位はｍｅｑ／ｋｇである。

【００３５】
【表２】

表2

		比較例			
		1	2	3	4
仕込み	原料 酸型リン脂質	牛脳ＰＳ	大豆ＰＳ	ＰＡ	大豆ＰＳ
	量（g）	０．５ｇ	５００ｇ	５００ｇ	１．９ｇ
	賦形剤 量（g）	ﾗｸﾄｱﾙﾌﾞﾐﾝ ０．０５ｇ	ｼｸﾛﾃﾞｷｽﾄﾘﾝ ２ｇ	ｶｾﾞｲﾝﾅﾄﾘｳﾑ ２１４０ｇ	ゼラチン １００ｇ
処理	均質化 処理の有無	あり	あり	あり	あり
	温度（℃）	３０	１０	６０	４５
	時間（分）	１５	１５	１５	１５
	乾燥方法	温風乾燥	ｽﾌﾟﾚｰﾄﾞﾗｲ	温風乾燥	ｽﾌﾟﾚｰﾄﾞﾗｲ
結果	収量ｇ 収率（%）	０．４８ ８７．３	４５９ ９１．４	２２５０ ８５．２	８９ ８７．３
	水分（%）	３．０	５．０	１０	３．５
	残存率（%）	５２	４７	４１	２０
	初期ＰＯＶ 経時試験後ＰＯＶ	５．９ １３９	４．１ ８９	８．１ ７７	９．１ ９１

【００３６】　　＊　＊【表３】

表3

		比較例		
		5	6	7
	原料 酸型リン脂質	牛脳ＰＳ	大豆ＰＳ	ＰＡ
結果	水分（%）	３	５	１０
	残存率（%）	１８	９	１９
	初期ＰＯＶ 経時試験後ＰＯＶ	０．１ １５９	０．３ １０１	０．１ ４９

【００３７】６カ月後の原料のホスファチジルセリンとホスファチジン酸および比較例１～４の場合には酸性リン脂質の残存率は少ないのに比べて、本発明の実施例１～４では酸性リン脂質の残存率が高いことがわかる。また、初期と６ヶ月後のＰＯＶを比較すると原料のホスファチジルセリンとホスファチジン酸および比較例１～４は共に値が大きくなり、酸化が進行しているのに対し、実施例１～４には酸化劣化がほとんど認められなかった。以上の結果から、本発明の実施例に示した酸性リン脂質は著しく安定していることが分かる。

[JP,2001-354680,A]

CLAIMS DETAILED DESCRIPTION TECHNICAL FIELD PRIOR ART EFFECT OF THE INVENTION TECHNICAL PROBLEM MEANS EXAMPLE

CLAIMS

[Claim(s)]

[Claim 1]A manufacturing method of high stability acidity phospholipid adding acid phospholipid and an excipient in water under 0-50 ** conditions, carrying out vacuum drying after uniformity in a method of manufacturing acid stable phospholipid by making acid phospholipid and an excipient living together, and making moisture into 1% or less.

[Claim 2]Acid phospholipid is phosphatidic acid or phosphatidylserine, A manufacturing method of the high stability acidity phospholipid according to claim 1 whose excipient is one sort chosen from a group which consists of carbohydrate, protein, peptide, and amino acid, or two sorts or more and whose quantity of an excipient is 0.01 to 100 weight section to acid phospholipid 1 weight section.

[Claim 3]A manufacturing method of the high stability acidity phospholipid according to claim 2 whose peptide whose protein which is an excipient is an excipient with lactalbumin or gelatin is egg white peptide.

[Claim 4]High stability acidity phospholipid whose rise of 10% or less and a peroxide number it is acid phospholipid obtained by a manufacturing method of claims 1-3, and a decrement of acid phospholipid is one or less during six-month preservation under conditions of storage temperature of 40 **, and 75% of relative humidity.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]This invention relates to extremely stable acidity phospholipid and a manufacturing method for the same.
[0002]
[Description of the Prior Art]Acid phospholipid is an acid thing of phospholipid. For example, the salt type thing of phosphatidylserine or phosphatidic acid is extracted from a bovine brain or vegetation, or. It has been manufactured by the base exchange reaction or hydrolysis which uses other phospholipid as a starting material (JP,63-36190,A, JP,63-36191,A, JP,63-36792,A, JP,2-79990,A, JP,4-171976,A). However, the advanced technology indicated above was difficult to take out phosphatidylserine or phosphatidic acid in the form of a salt after a reaction, and for acid type phospholipid not to exist in a nature easily.
[0003]On the other hand about acid phospholipid, various physiology activity is found out in recent years, physiology activity, such as a cerebral function improvement effect to which especially phosphatidylserine raises a learning effect, and an anti-stress effect, is found out, and the application to drugs, foodstuffs, etc. is expected. However, in the acid type state, these acidity phospholipid is chemically unstable and tended to receive quality degradation, such as oxidation and hydrolysis. For this reason, in the present commercial scene, acid phospholipid of the reagent grade was circulating in the state of frozen storage. However, as compared with the salt type thing, acid phospholipid was excellent in compatibility with other fats and oils, and had an advantage which is easy to perform product processing, and since it had different

physiology activity from a salt type, a method of manufacturing high stability acidity phospholipid was desired at ordinary temperature.

[0004]In JP,54-126206,A, it is in phospholipid content fats and oils, such as phosphatidylcholine. For example, although the copolymer of octadecylacrylamide N,N-dimethylacrylamide is added and the method of suppressing browning of phospholipid is indicated, About whether oxidation of acid phospholipid and hydrolysis are controlled by this method, it is unstated. The stabilization technique of films, such as phospholipid by covering with the water soluble polymer which consists of alkyl acrylamide and N-substitution acrylamide, is indicated by JP,8-53643,A. However, about whether oxidation of acid phospholipid and hydrolysis are controlled by this method, it is unstated. This art is membranous stabilization technique and there were problems, such as being inapplicable, in acid phospholipid of bulk.

[0005]In the Patent Publication Heisei No. 505403 [eight to] gazette, and the Patent Publication Heisei No. 502851 [11 to] gazette, the hydrophobic lipid containing phospholipid is added as an excipient using protein, such as cytokine, and the art which stabilizes protein is indicated. However, about whether oxidation of acid phospholipid and hydrolysis are controlled by this method, it is unstated. This art is proteinic stabilization technique and cannot be applied to acid phospholipid.

[0006]

[Problem(s) to be Solved by the Invention]The purpose of this invention is ordinary temperature and there is in providing acid stable phospholipid and a manufacturing method for the same.

[0007]

[Means for Solving the Problem]As a result of inquiring wholeheartedly in view of said problem, when acid phospholipid and an excipient were blended under a specific condition, this invention persons acquire knowledge which acid phospholipid stabilizes remarkably also at ordinary temperature, and came to complete this invention. That is, this invention is following [1] - [4].

[0008][1] A manufacturing method of high stability acidity phospholipid adding acid phospholipid and an excipient in water under 0-50 ** conditions, carrying out vacuum drying after uniformity in a method of manufacturing acid stable phospholipid by making acid phospholipid and an excipient living together, and making moisture into 1% or less.

[0009][2] Acid phospholipid is phosphatidic acid or phosphatidylserine, A manufacturing

method of high stability acidity phospholipid of the above [2] whose excipient is one sort chosen from a group which consists of carbohydrate, protein, peptide, and amino acid, or two sorts or more and whose acid phospholipid quantity of an excipient is 0.01 to 100 weight section to one weight section.

[0010][3] The aforementioned manufacturing method of high stability acidity phospholipid of [1] whose peptide whose protein which is an excipient is an excipient with lactalbumin or gelatin is egg white peptide.

[0011][4] High stability acidity phospholipid whose rise of 10% or less and a peroxide number it is acid phospholipid obtained by a manufacturing method of aforementioned [1] – [3], and a decrement of acid phospholipid is one or less during six-month preservation under conditions of storage temperature of 40 **, and 75% of relative humidity.

[0012]

[Embodiment of the Invention]As for the phospholipid used for this invention, lecithin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, lysolecithin, etc. are mentioned, for example. Phosphatidylserine and phosphatidic acid are mentioned preferably especially. what extracted the phospholipid to be used from natural resources -- or although compounded, it can be used regardless of how. A commercial thing or the thing prepared by the publicly known method may be used. For example, it may use independently, and two or more sorts may be combined and what was extracted from a soybean, a defatted soybean, an egg yolk, etc. may be used. It is not limited in particular for the origin of an animal, vegetation, etc. For example, it is also possible to use what was obtained from a soybean lecithin, defatted soybean lecithin, yolk lecithin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, lysolecithin, etc. by a base exchange reaction or hydrolysis. As acid phospholipid used for this invention, it is obtained by processing salt type phospholipid from acid. As the aforementioned acid, formic acid, acetic acid, succinic acid, malic acid, fumaric acid, maleic acid, citrate, chloride, sulfuric acid, nitric acid, phosphoric acid, phytic acid, etc. are mentioned, for example. It can use combining one sort chosen from these, or two sorts or more. It is more preferred to use organic acid, such as chloride, sulfuric acid, nitric acid, inorganic acid of phosphoric acid, and phytic acid, from a point of the removing effect of a positive ion.

[0013]It is more desirable to adjust a buffer solution solution, for example, formic

acid–sodium formate buffer solution, glycine chloride buffer solution, acetic acid–sodium acetate buffer solution, citrate–sodium–acid–citrate buffer solution, a phosphate buffer solution, etc. to predetermined pH, and to use them. The addition of acid or buffer solution sets more preferably 1–100 mol of acid [15–60 mol of] as 20–50 mol to 1 mol of salt type phospholipid. Less than 1 mol of an addition is not enough as the effect of acid, and it cannot manufacture high grade acidity phospholipid. If the addition of acid exceeds 100 mol, since side reactions, such as hydrolysis, will arise, it is not desirable. 0–50 ** of temperature [5–30 ** of] of the acid added to the system of reaction is 7–20 ** more preferably. Handling becomes difficult, in order that the viscosity of acid may rise at less than 0 ** or temperature may solidify. If the temperature of acid exceeds 50 **, since side reactions, such as hydrolysis, will arise, it is not desirable. For example, as a commercial item of acid phospholipid, bovine brain origin phosphatidylserine (98% of purity: sigma company products), soybean origin phosphatidylserine (98% of purity: sigma company products), dipalmitoyl phosphatidic acid (99% of purity: sigma company products), etc. are mentioned as a desirable thing.

[0014]Or it is different species and the constituent fatty acids of the phospholipid used for this invention are fatty acid of the saturation of the carbon numbers 8–24, or an unsaturation. For example, caproic acid, capric acid, lauric acid, myristic acid, stearic acid, Behenic acid, arachidic acid, palmitoleic acid, oleic acid, linolic acid, alpha and gamma–linolenic acid, erucic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, tetracosa tetraenoic acid, etc. are mentioned. Especially, oleic acid, linolic acid, eicosapentaenoic acid, and docosahexaenoic acid are preferred from the field of physiology activity.

[0015]As an excipient used for this invention, carbohydrate, protein, peptide, and amino acid are mentioned, for example. As the aforementioned carbohydrate, polysaccharide, such as disaccharides, such as monosaccharides, such as starch, dextrin, cellulose and its hydrolyzate, grape sugar, and fructose, malt sugar, and milk sugar, gum arabic, xanthan gum, locust bean gum, and pullulan, etc. are mentioned, for example. It may use combining these one sort or two sorts or more. For example, lactose (RAPURINO products), SD–20 (cyclodextrin: Ensuiko Sugar Refining Co., Ltd. products), pineapple DEKKUSU (dextrin: Matsutani Chemicals products), etc. are mentioned as a desirable thing as a commercial item.

[0016]The protein of an animal and a vegetable system is used as the aforementioned

protein. As an animal protein, casein, collagen, a whey protein, egg protein, fish protein, etc. are mentioned, for example. As vegetable albumen, soybean protein, the quality of wheat protein, maize protein, etc. are mentioned. As the aforementioned peptide, the decomposition product of the aforementioned protein or its salt is mentioned, for example, egg white peptide, fish peptide, lactalbumin peptide, etc. are mentioned. As the aforementioned amino acid, the decomposition product of the aforementioned peptide or various amino acid can be used. Also in this, gelatin, egg white peptide, lactalbumin, caseinate, etc. are preferably mentioned from the field of supply stability. As a commercial item, for example, the low molecule collagen powder SPH (SNOW BRAND FOOD CO., LTD. products), The RAKUPU rhodan 80 (lactalbumin: MD foods yne GUREDIENTSU Japan products), The magnesium caseinate S (DMV Japan products), CE90F (whey peptide: DMV Japan products), EP-3 (egg-white peptide: Kewpie products), etc. are mentioned as a desirable thing.

[0017]The amount of the excipient used used by this invention is 0.5 to 10 weight section more preferably 0.2 to 50 weight section 0.01 to 100 weight section to acid phospholipid 1 weight section used as a raw material. Even if it makes the addition of an excipient more than 100 weight sections to acid phospholipid 1 weight section, the improved effect of the remarkable stabilization corresponding to it is not accepted, and the stabilization effect by an excipient is not acquired in less than 0.01 weight section.

[0018]The gestalt which carries out this invention makes acid phospholipid include with an excipient by dissolving the excipient of the range of 0.01 to 100 weight section in water in a stirred tank to acid phospholipid 1 weight section used as a raw material, adding the powder of acid phospholipid subsequently and making it uniform. Inclusion is performed in 0-50 **. If it becomes lower than 0 ** accompanied by producing the denaturation of the protein which is an excipient if it becomes higher than 50 **, and disassembly of acid phospholipid, the aqueous phase will solidify. When including, homogeneous machines, such as a homogenizer, polyTRON, a Micro fluidizer, and a homomixer, can be used together. Degradation of the fatty acid contained in phospholipid can be prevented by performing a reaction under nitrogen flowing back. Then, it dries with vacuum dryers, such as a vacuum belt dryer and freeze-drying, and the moisture in a final product is made into 1% or less.

[0019]As for the acid phospholipid manufactured by the method of this invention, the decrement of acid phospholipid raises remarkably the stabilization for which the rise of

a peroxide number can attain one or less 10% or less during six-month preservation under the conditions of the storage temperature of 40 **, and 75% of relative humidity.

[0020]

[Effect of the Invention]The manufacturing method of high stability acidity phospholipid of this invention, Under specific conditions, acid phospholipid of a raw material and a specific excipient can be added, stabilization can be attained, acid phospholipid and an excipient can be added in water under 0-50 ** conditions, and acid phospholipid stable by the easy method of carrying out vacuum drying and making moisture 1% or less after uniformity, can be obtained. By stabilizing the acid phospholipid which has various physiology activity, quality can be maintained and it can apply to health food, drugs, etc. industrially for a long period of time.

[0021]

[Example]This invention is explained based on an example. The measuring method and valuation method which were used are shown.

1. It carried out using the fixed-quantity high performance chromatography (made in Gilson, model model 303) of phospholipid. A silica gel column (diameter [of 4.6 mm] x250 mm in length) is used for a stationary phase, moreover -- a mobile phase -- acetonitrile: -- methanol: -- detection measures the absorption in the ultraviolet-rays UV wavelength of 202 nm using the mixed solvent of 10mM ammonium-dihydrogenphosphate =612:289:100 -- a fixed quantity -- the bottom.

2. POV used as the index of measurement oxidation of a peroxide number (POV) was measured according to the standard fats-and-oils assay method 2.4.12-86 of Japanese oil recovery study association establishment.

3. The moisture in the test portion of moisture was measured using curl Fischer moisture meter, Mitsubishi Chemical make, and model CA-06.

4. Evaluation of the valuation method stability of stability was an opened condition about the powder sample, measured the content of acid phospholipid with high performance chromatography (HPLC) after preservation for six months within 40 ** and the thermostat of 75% of relative humidity, and searched for the survival rate.

5. By the opened condition, measurement of the peroxide number (POV) was performed for the first stage and a powder sample after preservation for six months within 40 ** and the thermostat of 75% of relative humidity by the method of the oxidation stability above.

[0022]Mix the water 3g with the example 1 lactalbumin 0.05g, and it was made to stir and dissolve with an ultrasonic washing machine, and it uniformed for 15 minutes using polyTRON (made by KINEMACHIKA) at 30 ** after adding 0.5 g of acid bovine brain origin phosphatidylserine (98% of purity: sigma company article) subsequently, performing a nitrogen purge. It freeze-dried after uniformity and the powder 0.54g was obtained. It was 0.2% as a result of measuring the moisture of a sample by the aforementioned method.

[0023]500 g of example 2 cyclodextrin was put into the water 3000g, and it dissolved by the propeller agitator, and after adding 500 g of acid soybean origin phosphatidylserine (98% of purity: sigma company article) subsequently, while the homomixer performed the nitrogen purge at 10 **, it uniformed for 15 minutes. Vacuum belt desiccation was performed after the reaction and the powder 990g was obtained. It was 0.1% as a result of measuring moisture similarly.

[0024]The example 3 casein sodium 2140g and the water 5000g were stirred and dissolved in the homomixer, and it uniformed for 15 minutes by the homomixer at 1 ** after adding 500 g of acid dipalmitoyl phosphatidic acid (99% of purity: sigma company article) subsequently. It freeze-dried after uniformity and the powder 2580g was obtained. It was 0.3% as a result of measuring the moisture of a sample.

[0025]100 g of example 4 gelatin, and 800g50 ** of water -- warming -- it was made to dissolve by after propeller churning, after cooling at 45 **, the nitrogen purge was performed after adding 1.9 g of soybean origin phosphatidylserine (98% of purity: sigma company article), and it uniformed for 15 minutes by the SONIFA year (made in Branson). It freeze-dried after the reaction and the powder 101g was obtained. It was 0.1% as a result of measuring the moisture of a sample similarly.

[0026]Mix the water 3g with the comparative example 1 lactalbumin 0.05g, and it was made to stir and dissolve with an ultrasonic washing machine, and subsequently it uniformed for 15 minutes using polyTRON (KINEMACHIKA) at 30 ** after adding 0.5 g of acid bovine brain origin phosphatidylserine (98% of purity: sigma company products), performing a nitrogen purge. Warm air desiccation was performed after uniformity and the powder 0.48g was obtained. It was 3.0% as a result of measuring the moisture of a sample similarly.

[0027]2 g of comparative example 2 cyclodextrin was put into the water 3000g, and it dissolved by the propeller agitator, and after adding 500 g of acid soybean origin

phosphatidylserine (98% of purity: sigma company article) subsequently, while the homomixer performed the nitrogen purge at 10 **, it uniformed for 15 minutes. It spray-dried after the reaction and the powder 459g was obtained. It was 5.0% as a result of measuring the moisture of a sample similarly.

[0028]The comparative example 3 casein sodium 2140g and the water 5000g were stirred and dissolved in the homomixer, and it uniformed for 15 minutes by the homomixer at 60 ** after adding 500 g of acid dipalmitoyl phosphatidic acid (99% of purity: sigma company products) subsequently. Warm air desiccation was performed after uniformity and the powder 2250g was obtained. It was 10% as a result of measuring the moisture of a sample similarly.

[0029]100 g of comparative example 4 gelatin, and 800g50 ** of water -- warming -- it was made to dissolve by after propeller churning, and after cooling at 45 **, it agitated for 15 minutes by propeller churning further after adding 1.9 g of acid soybean origin phosphatidylserine (98% of purity: sigma company products). It spray-dried after the reaction and the powder of the powder 89g was obtained. It was 3.5% as a result of measuring the moisture of a sample similarly.

[0030]The survival rate was searched for in the formula measuring the ullage of phosphatidylserine or phosphatidic acid in HPLC and in which showing below the powder obtained by example of examination 1 Examples 1-4, and the comparative examples 1-4 after preservation for six months within 40 ** and the thermostat of 75% of relative humidity.

survival-rate (%) =(acid phospholipid content of acid phospholipid content / first stage six months after) x100 -- the result is shown in Tables 1 and 2.

[0031]Measurement of POV was performed for the powder obtained by example of examination 2 Examples 1-4, and the comparative examples 1-4 after preservation for six months within 40 ** and the thermostat of 75% of relative humidity. POV six months after an initial value was shown in Tables 1 and 2.

[0032]Stability was evaluated for the phosphatidylserine and phosphatidic acid of example of examination 3 raw material like the method of the aforementioned example of an examination. It is shown in Table 3 by making a result into the comparative examples 5-7.

[0033]

[Table 1]

表1

		実施例			
		1	2	3	4
仕込み	原料 酸型リン脂質 量（g）	牛脳ＰＳ ０．５ｇ	大豆ＰＳ ５００ｇ	ＰＡ ５００ｇ	大豆ＰＳ １．９ｇ
	賦形剤 量（g）	ﾗｸﾄｱﾙﾌﾞﾐﾝ ０．０５ｇ	ｼｸﾛﾃﾞｷｽﾄﾘﾝ ５００ｇ	ｶｾﾞｲﾝｻﾝﾅﾄﾘｳﾑ ２１４０ｇ	ゼラチン １００ｇ
処理	均質化 処理の有無 温度（℃）	あり ３０	あり １０	あり １	あり ４５
	時間（分）	１５	１５	１５	１５
	乾燥方法	真空凍結乾燥	真空ﾍﾞﾙﾄ乾燥	真空凍結乾燥	真空ﾍﾞﾙﾄ乾燥
結果	収量ｇ 収率（%）	０．５４ ９８．２	９９０ ９９．０	２５８０ ９９．７	１０１ ９９．２
	水分（%）	０．２	０．１	０．３	０．１
	残存率（%）	９９	９６	９８	９８
	初期ＰＯＶ* 経時試験後ＰＯＶ	０．１ ０．３	０．３ ０．５	０．１ ０．４	０．３ ０．４

[0034]The cable address used into front is as follows.

PS; phosphatidylserine (acid type)

PA; phosphatidic acid.

The unit of POV is meq/kg.

[0035]

[Table 2]

表2

		比較例			
		1	2	3	4
仕込み	原料 酸型リン脂質 量（g）	牛脳ＰＳ 0．5ｇ	大豆ＰＳ ５００ｇ	ＰＡ ５００ｇ	大豆ＰＳ 1．9ｇ
	賦形剤 量（g）	ﾗｸﾄｱﾙﾌﾞﾐﾝ 0．05ｇ	ｼｸﾛﾃﾞｷｽﾄﾘﾝ 2ｇ	ｶｾﾞｲﾝﾅﾄﾘｳﾑ ２１４０ｇ	ゼラチン １００ｇ
処理	均質化 処理の有無 温度（℃）	あり ３０	あり １０	あり ６０	あり ４５
	時間（分）	１５	１５	１５	１５
	乾燥方法	温風乾燥	ｽﾌﾟﾚｰﾄﾞﾗｲ	温風乾燥	ｽﾌﾟﾚｰﾄﾞﾗｲ
結果	収量g 収率（%）	0．４８ ８７．３	４５９ ９１．４	２２５０ ８５．２	８９ ８７．３
	水分（%）	３．０	５．０	１０	３．５
	残存率（%）	５２	４７	４１	２０
	初期ＰＯＶ 経時試験後ＰＯＶ	５．９ １３９	４．１ ８９	８．１ ７７	９．１ ９１

[0036]

[Table 3]

表3

		比較例		
		5	6	7
	原料 酸型リン脂質	牛脳ＰＳ	大豆ＰＳ	ＰＡ
結果	水分（%）	３	５	１０
	残存率（%）	１３	９	１９
	初期ＰＯＶ 経時試験後ＰＯＶ	０．１ １５９	０．３ １０１	０．１ ４９

[0037]In the case of the phosphatidylserine of the raw material of six months after, phosphatidic acid, and the comparative examples 1-4, the survival rate of acid phospholipid is understood that the survival rate of acid phospholipid is high in Examples 1-4 of this invention compared with few things. When POV of the first stage and six months after was compared, the value became large and, as for both the

phosphatidylserine, phosphatidic acid, and comparative examples 1-4 of a raw material, oxidation degradation was hardly observed in Examples 1-4 to oxidation advancing. The above result shows that the acid phospholipid shown in the example of this invention is remarkably stable.

[Translation done.]

TECHNICAL FIELD

[Field of the Invention]This invention relates to extremely stable acidity phospholipid and a manufacturing method for the same.

[Translation done.]

PRIOR ART

[Description of the Prior Art]Acid phospholipid is an acid thing of phospholipid. For example, the salt type thing of phosphatidylserine or phosphatidic acid is extracted from a bovine brain or vegetation, or. It has been manufactured by the base exchange reaction or hydrolysis which uses other phospholipid as a starting material (JP,63-36190,A, JP,63-36191,A, JP,63-36792,A, JP,2-79990,A, JP,4-171976,A). However, the advanced technology indicated above was difficult to take out phosphatidylserine or phosphatidic acid in the form of a salt after a reaction, and for acid type phospholipid not to exist in a nature easily.

[0003]On the other hand about acid phospholipid, various physiology activity is found out in recent years, physiology activity, such as a cerebral function improvement effect to which especially phosphatidylserine raises a learning effect, and an anti-stress effect, is found out, and the application to drugs, foodstuffs, etc. is expected. However, in the acid type state, these acidity phospholipid is chemically unstable and tended to

receive quality degradation, such as oxidation and hydrolysis. For this reason, in the present commercial scene, acid phospholipid of the reagent grade was circulating in the state of frozen storage. However, as compared with the salt type thing, acid phospholipid was excellent in compatibility with other fats and oils, and had an advantage which is easy to perform product processing, and since it had different physiology activity from a salt type, a method of manufacturing high stability acidity phospholipid was desired at ordinary temperature.

[0004]In JP,54-126206,A, it is in phospholipid content fats and oils, such as phosphatidylcholine. For example, although the copolymer of octadecylacrylamide N,N-dimethylacrylamide is added and the method of suppressing browning of phospholipid is indicated, About whether oxidation of acid phospholipid and hydrolysis are controlled by this method, it is unstated. The stabilization technique of films, such as phospholipid by covering with the water soluble polymer which consists of alkyl acrylamide and N-substitution acrylamide, is indicated by JP,8-53643,A. However, about whether oxidation of acid phospholipid and hydrolysis are controlled by this method, it is unstated. This art is membranous stabilization technique and there were problems, such as being inapplicable, in acid phospholipid of bulk.

[0005]In the Patent Publication Heisei No. 505403 [eight to] gazette, and the Patent Publication Heisei No. 502851 [11 to] gazette, the hydrophobic lipid containing phospholipid is added as an excipient using protein, such as cytokine, and the art which stabilizes protein is indicated. However, about whether oxidation of acid phospholipid and hydrolysis are controlled by this method, it is unstated. This art is proteinic stabilization technique and cannot be applied to acid phospholipid.

[Translation done.]

EFFECT OF THE INVENTION

[Effect of the Invention]The manufacturing method of high stability acidity phospholipid of this invention, Under specific conditions, acid phospholipid of a raw material and a specific excipient can be added, stabilization can be attained, acid phospholipid and an

excipient can be added in water under 0–50 ** conditions, and acid phospholipid stable by the easy method of carrying out vacuum drying and making moisture 1% or less after uniformity, can be obtained. By stabilizing the acid phospholipid which has various physiology activity, quality can be maintained and it can apply to health food, drugs, etc. industrially for a long period of time.

[Translation done.]

TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention]The purpose of this invention is ordinary temperature and there is in providing acid stable phospholipid and a manufacturing method for the same.

[Translation done.]

MEANS

[Means for Solving the Problem]As a result of inquiring wholeheartedly in view of said problem, when acid phospholipid and an excipient were blended under a specific condition, this invention persons acquire knowledge which acid phospholipid stabilizes remarkably also at ordinary temperature, and came to complete this invention. That is, this invention is following [1] – [4].

[0008][1] A manufacturing method of high stability acidity phospholipid adding acid phospholipid and an excipient in water under 0–50 ** conditions, carrying out vacuum drying after uniformity in a method of manufacturing acid stable phospholipid by making acid phospholipid and an excipient living together, and making moisture into 1% or less.

[0009][2] Acid phospholipid is phosphatidic acid or phosphatidylserine, A manufacturing method of high stability acidity phospholipid of the above [2] whose excipient is one

sort chosen from a group which consists of carbohydrate, protein, peptide, and amino acid, or two sorts or more and whose acid phospholipid quantity of an excipient is 0.01 to 100 weight section to one weight section.

[0010][3] The aforementioned manufacturing method of high stability acidity phospholipid of [1] whose peptide whose protein which is an excipient is an excipient with lactalbumin or gelatin is egg white peptide.

[0011][4] High stability acidity phospholipid whose rise of 10% or less and a peroxide number it is acid phospholipid obtained by a manufacturing method of aforementioned [1] – [3], and a decrement of acid phospholipid is one or less during six-month preservation under conditions of storage temperature of 40 **, and 75% of relative humidity.

[0012]

[Embodiment of the Invention]As for the phospholipid used for this invention, lecithin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, lysolecithin, etc. are mentioned, for example. Phosphatidylserine and phosphatidic acid are mentioned preferably especially. what extracted the phospholipid to be used from natural resources -- or although compounded, it can be used regardless of how. A commercial thing or the thing prepared by the publicly known method may be used. For example, it may use independently, and two or more sorts may be combined and what was extracted from a soybean, a defatted soybean, an egg yolk, etc. may be used. It is not limited in particular for the origin of an animal, vegetation, etc. For example, it is also possible to use what was obtained from a soybean lecithin, defatted soybean lecithin, yolk lecithin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, lysolecithin, etc. by a base exchange reaction or hydrolysis. As acid phospholipid used for this invention, it is obtained by processing salt type phospholipid from acid. As the aforementioned acid, formic acid, acetic acid, succinic acid, malic acid, fumaric acid, maleic acid, citrate, chloride, sulfuric acid, nitric acid, phosphoric acid, phytic acid, etc. are mentioned, for example. It can use combining one sort chosen from these, or two sorts or more. It is more preferred to use organic acid, such as chloride, sulfuric acid, nitric acid, inorganic acid of phosphoric acid, and phytic acid, from a point of the removing effect of a positive ion.

[0013]It is more desirable to adjust a buffer solution solution, for example, formic acid-sodium formate buffer solution, glycine chloride buffer solution, acetic

acid–sodium acetate buffer solution, citrate–sodium–acid–citrate buffer solution, a phosphate buffer solution, etc. to predetermined pH, and to use them. The addition of acid or buffer solution sets more preferably 1–100 mol of acid [15–60 mol of] as 20–50 mol to 1 mol of salt type phospholipid. Less than 1 mol of an addition is not enough as the effect of acid, and it cannot manufacture high grade acidity phospholipid. If the addition of acid exceeds 100 mol, since side reactions, such as hydrolysis, will arise, it is not desirable. 0–50 ** of temperature [5–30 ** of] of the acid added to the system of reaction is 7–20 ** more preferably. Handling becomes difficult, in order that the viscosity of acid may rise at less than 0 ** or temperature may solidify. If the temperature of acid exceeds 50 **, since side reactions, such as hydrolysis, will arise, it is not desirable. For example, as a commercial item of acid phospholipid, bovine brain origin phosphatidylserine (98% of purity: sigma company products), soybean origin phosphatidylserine (98% of purity: sigma company products), dipalmitoyl phosphatidic acid (99% of purity: sigma company products), etc. are mentioned as a desirable thing.

[0014]Or it is different species and the constituent fatty acids of the phospholipid used for this invention are fatty acid of the saturation of the carbon numbers 8–24, or an unsaturation. For example, caproic acid, capric acid, lauric acid, myristic acid, stearic acid, Behenic acid, arachidic acid, palmitoleic acid, oleic acid, linolic acid, alpha and gamma–linolenic acid, erucic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, tetracosa tetraenoic acid, etc. are mentioned. Especially, oleic acid, linolic acid, eicosapentaenoic acid, and docosahexaenoic acid are preferred from the field of physiology activity.

[0015]As an excipient used for this invention, carbohydrate, protein, peptide, and amino acid are mentioned, for example. As the aforementioned carbohydrate, polysaccharide, such as disaccharides, such as monosaccharides, such as starch, dextrin, cellulose and its hydrolyzate, grape sugar, and fructose, malt sugar, and milk sugar, gum arabic, xanthan gum, locust bean gum, and pullulan, etc. are mentioned, for example. It may use combining these one sort or two sorts or more. For example, lactose (RAPURINO products), SD–20 (cyclodextrin: Ensuiko Sugar Refining Co., Ltd. products), pineapple DEKKUSU (dextrin: Matsutani Chemicals products), etc. are mentioned as a desirable thing as a commercial item.

[0016]The protein of an animal and a vegetable system is used as the aforementioned protein. As an animal protein, casein, collagen, a whey protein, egg protein, fish protein,

etc. are mentioned, for example. As vegetable albumen, soybean protein, the quality of wheat protein, maize protein, etc. are mentioned. As the aforementioned peptide, the decomposition product of the aforementioned protein or its salt is mentioned, for example, egg white peptide, fish peptide, lactalbumin peptide, etc. are mentioned. As the aforementioned amino acid, the decomposition product of the aforementioned peptide or various amino acid can be used. Also in this, gelatin, egg white peptide, lactalbumin, caseinate, etc. are preferably mentioned from the field of supply stability. As a commercial item, for example, the low molecule collagen powder SPH (SNOW BRAND FOOD CO., LTD. products), The RAKUPU rhodan 80 (lactalbumin: MD foods yne GUREDIENTSU Japan products), The magnesium caseinate S (DMV Japan products), CE90F (whey peptide: DMV Japan products), EP-3 (egg-white peptide: Kewpie products), etc. are mentioned as a desirable thing.

[0017]The amount of the excipient used used by this invention is 0.5 to 10 weight section more preferably 0.2 to 50 weight section 0.01 to 100 weight section to acid phospholipid 1 weight section used as a raw material. Even if it makes the addition of an excipient more than 100 weight sections to acid phospholipid 1 weight section, the improved effect of the remarkable stabilization corresponding to it is not accepted, and the stabilization effect by an excipient is not acquired in less than 0.01 weight section.

[0018]The gestalt which carries out this invention makes acid phospholipid include with an excipient by dissolving the excipient of the range of 0.01 to 100 weight section in water in a stirred tank to acid phospholipid 1 weight section used as a raw material, adding the powder of acid phospholipid subsequently and making it uniform. Inclusion is performed in 0-50 **. If it becomes lower than 0 ** accompanied by producing the denaturation of the protein which is an excipient if it becomes higher than 50 **, and disassembly of acid phospholipid, the aqueous phase will solidify. When including, homogeneous machines, such as a homogenizer, polyTRON, a Micro fluidizer, and a homomixer, can be used together. Degradation of the fatty acid contained in phospholipid can be prevented by performing a reaction under nitrogen flowing back. Then, it dries with vacuum dryers, such as a vacuum belt dryer and freeze-drying, and the moisture in a final product is made into 1% or less.

[0019]As for the acid phospholipid manufactured by the method of this invention, the decrement of acid phospholipid raises remarkably the stabilization for which the rise of a peroxide number can attain one or less 10% or less during six-month preservation

under the conditions of the storage temperature of 40 **, and 75% of relative humidity.

[Translation done.]

EXAMPLE

[Example]This invention is explained based on an example. The measuring method and valuation method which were used are shown.

1. It carried out using the fixed-quantity high performance chromatography (made in Gilson, model model 303) of phospholipid. A silica gel column (diameter [of 4.6 mm] x250 mm in length) is used for a stationary phase, moreover -- a mobile phase -- acetonitrile: -- methanol: -- detection measures the absorption in the ultraviolet-rays UV wavelength of 202 nm using the mixed solvent of 10mM ammonium-dihydrogenphosphate =612:289:100 -- a fixed quantity -- the bottom.

2. POV used as the index of measurement oxidation of a peroxide number (POV) was measured according to the standard fats-and-oils assay method 2.4.12-86 of Japanese oil recovery study association establishment.

3. The moisture in the test portion of moisture was measured using curl Fischer moisture meter, Mitsubishi Chemical make, and model CA-06.

4. Evaluation of the valuation method stability of stability was an opened condition about the powder sample, measured the content of acid phospholipid with high performance chromatography (HPLC) after preservation for six months within 40 ** and the thermostat of 75% of relative humidity, and searched for the survival rate.

5. By the opened condition, measurement of the peroxide number (POV) was performed for the first stage and a powder sample after preservation for six months within 40 ** and the thermostat of 75% of relative humidity by the method of the oxidation stability above.

[0022]Mix the water 3g with the example 1 lactalbumin 0.05g, and it was made to stir and dissolve with an ultrasonic washing machine, and it uniformed for 15 minutes using polyTRON (made by KINEMACHIKA) at 30 ** after adding 0.5 g of acid bovine brain origin phosphatidylserine (98% of purity: sigma company article) subsequently,

performing a nitrogen purge. It freeze-dried after uniformity and the powder 0.54g was obtained. It was 0.2% as a result of measuring the moisture of a sample by the aforementioned method.

[0023]500 g of example 2 cyclodextrin was put into the water 3000g, and it dissolved by the propeller agitator, and after adding 500 g of acid soybean origin phosphatidylserine (98% of purity: sigma company article) subsequently, while the homomixer performed the nitrogen purge at 10 **, it uniformed for 15 minutes. Vacuum belt desiccation was performed after the reaction and the powder 990g was obtained. It was 0.1% as a result of measuring moisture similarly.

[0024]The example 3 casein sodium 2140g and the water 5000g were stirred and dissolved in the homomixer, and it uniformed for 15 minutes by the homomixer at 1 ** after adding 500 g of acid dipalmitoyl phosphatidic acid (99% of purity: sigma company article) subsequently. It freeze-dried after uniformity and the powder 2580g was obtained. It was 0.3% as a result of measuring the moisture of a sample.

[0025]100 g of example 4 gelatin, and 800g50 ** of water -- warming -- it was made to dissolve by after propeller churning, after cooling at 45 **, the nitrogen purge was performed after adding 1.9 g of soybean origin phosphatidylserine (98% of purity: sigma company article), and it uniformed for 15 minutes by the SONIFA year (made in Branson). It freeze-dried after the reaction and the powder 101g was obtained. It was 0.1% as a result of measuring the moisture of a sample similarly.

[0026]Mix the water 3g with the comparative example 1 lactalbumin 0.05g, and it was made to stir and dissolve with an ultrasonic washing machine, and subsequently it uniformed for 15 minutes using polyTRON (KINEMACHIKA) at 30 ** after adding 0.5 g of acid bovine brain origin phosphatidylserine (98% of purity: sigma company products), performing a nitrogen purge. Warm air desiccation was performed after uniformity and the powder 0.48g was obtained. It was 3.0% as a result of measuring the moisture of a sample similarly.

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survival-rate (%) =(acid phospholipid content of acid phospholipid content / first stage six months after) x100 -- the result is shown in Tables 1 and 2.

[0031]Measurement of POV was performed for the powder obtained by example of examination 2 Examples 1-4, and the comparative examples 1-4 after preservation for six months within 40 ** and the thermostat of 75% of relative humidity. POV six months after an initial value was shown in Tables 1 and 2.

[0032]Stability was evaluated for the phosphatidylserine and phosphatidic acid of example of examination 3 raw material like the method of the aforementioned example of an examination. It is shown in Table 3 by making a result into the comparative examples 5-7.

[0033]

[Table 1]

表1

		実施例			
		1	2	3	4
仕込み	原料 酸型リン脂質量（g）	牛脳ＰＳ ０．５ｇ	大豆ＰＳ ５００ｇ	ＰＡ ５００ｇ	大豆ＰＳ １．９ｇ
	賦形剤量（g）	ﾗｸﾄｱﾙﾌﾞﾐﾝ ０．０５ｇ	ｼｸﾛﾃﾞｷｽﾄﾘﾝ ５００ｇ	ｶｾﾞｲﾝﾅﾄﾘｳﾑ ２１４０ｇ	ゼラチン １００ｇ
処理	均質化処理の有無 温度（℃）	あり ３０	あり １０	あり １	あり ４５
	時間（分）	１５	１５	１５	１５
	乾燥方法	真空凍結乾燥	真空ﾍﾞﾙﾄ乾燥	真空凍結乾燥	真空ﾍﾞﾙﾄ乾燥
結果	収量ｇ 収率（%）	０．５４ ９８．２	９９０ ９９．０	２５８０ ９９．７	１０１ ９９．２
	水分（%）	０．２	０．１	０．３	０．１
	残存率（%）	９９	９６	９８	９８
	初期ＰＯＶ* 経時試験後ＰＯＶ	０．１ ０．３	０．３ ０．５	０．１ ０．４	０．３ ０．４

[0034]The cable address used into front is as follows.

PS; phosphatidylserine (acid type)

PA; phosphatidic acid.

The unit of POV is meq/kg.

[0035]

[Table 2]

表2

		比較例			
		1	2	3	4
仕込み	原料 酸型リン脂質 量（g）	牛脳ＰＳ ０．５ｇ	大豆ＰＳ ５００ｇ	ＰＡ ５００ｇ	大豆ＰＳ １．９ｇ
	賦形剤 量（g）	ﾗｸﾄｱﾙﾌﾞﾐﾝ ０．０５ｇ	ｼｸﾛﾃﾞｷｽﾄﾘﾝ ２ｇ	ｶｾﾞｲﾝﾅﾄﾘｳﾑ ２１４０ｇ	ゼラチン １００ｇ
処理	均質化 処理の有無 温度（℃）	あり ３０	あり １０	あり ６０	あり ４５
	時間（分）	１５	１５	１５	１５
	乾燥方法	温風乾燥	ｽﾌﾟﾚｰﾄﾞﾗｲ	温風乾燥	ｽﾌﾟﾚｰﾄﾞﾗｲ
結果	収量ｇ 収率（%）	０．４８ ８７．３	４５９ ９１．４	２２５０ ８５．２	８９ ８７．３
	水分（%）	３．０	５．０	１０	３．５
	残存率（%）	５２	４７	４１	２０
	初期ＰＯＶ 経時試験後ＰＯＶ	５．９ １３９	４．１ ８９	８．１ ７７	９．１ ９１

[0036]

[Table 3]

表3

		比較例		
		5	6	7
	原料 酸型リン脂質	牛脳ＰＳ	大豆ＰＳ	ＰＡ
結果	水分（%）	３	５	１０
	残存率（%）	１３	９	１９
	初期ＰＯＶ 経時試験後ＰＯＶ	０．１ １５９	０．３ １０１	０．１ ４９

[0037]In the case of the phosphatidylserine of the raw material of six months after, phosphatidic acid, and the comparative examples 1-4, the survival rate of acid phospholipid is understood that the survival rate of acid phospholipid is high in Examples 1-4 of this invention compared with few things. When POV of the first stage and six months after was compared, the value became large and, as for both the

phosphatidylserine, phosphatidic acid, and comparative examples 1–4 of a raw material, oxidation degradation was hardly observed in Examples 1–4 to oxidation advancing. The above result shows that the acid phospholipid shown in the example of this invention is remarkably stable.

[Translation done.]